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SOME OBSERVATIONS UPON THE STATUS OF THE GENERIC NAME SELENOMONAS PROWAZEK

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The following observations are in favour of Draft Proposal Alternative 2 and against Alternative 1 (Judicial Commission, 1955). A good case can be made out for regarding the generic name Selenomastix, conferred by Woodcock and Lapage (1913) on certain goat rumen organisms, as invalid by reason of uncertainty. As all later students of rumen microbiology have confirmed (cf. McGaughey and Sellers, 1948) there were at least two morphologically distinct organisms described by Woodcock and Lapage, to which the name Selenomastix was indiscriminately applied by these authors; and although they usually occur together in the rumen, no one has yet shown that a relationship of any sort exists between them. They are in fact very different in many respects. Thus, one resembles Selenomonas palpitans from the guinea pig caecum (see Lessel and Breed, 1954) in being crescent-shaped and motile by a group of lateral flagella; the other is purely oval in shape and is never flagellated. Nevertheless it is indisputably motile upon occasion. Unlike the crescents, the ovals have a visible cell wall or envelope. The yeast-like glycogen-storing organisms studied by Quin (1943) consisted entirely of the latter, as his description and photomicrograph clearly show. Since the excellent photomicrographs of the caecal Selenomonas palpitans in Lessel and Breed (1954) show only a crescent form, never an oval, there is no warrant by analogy for assuming that the rumen ovals might be one stage in the life cycle of an organism which may also exist at another stage in its life history as flagellated crescents. In fact Woodcock and Lapage noted that one form might be rare and the other plentiful in a given rumen. In the rumen, therefore, it would be safer to regard them as two distinct organisms and since the rumen crescents might

legitimately be placed in the genus Selenomonas, it is arguable that the rumen ovals, studied by Quin and called by him Schizosaccharomyces ovis, might legitimately be named Selenomastix ruminantium (Woodcoek and Lapage, 1913) since these authors did apply that name to the rumen ovals as well as the crescents. Whatever else the ovals may be they are certainly not yeasts (see van der Westhuizen and Oxford, 1950, and pace McGaughey and Sellers (1948). I would say that they have not yet validly been named. If subsequent investigation should reveal a real relationship between the rumen crescents and ovals, the above conclusion would, of course, need revision.

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SPECIFIC EPITHET FOR THE TYPE SPECIES OF STAPHYLOCOCCUS

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The choice of a specific epithet for the type species of the genus Staphylococcus has most often been based on the personal preference of an author, sometimes coupled with a sense of appropriateness, rather than on a study of the early literature. Although the majority of authors used Staphylococcus aureus, many, myself included, argued that as pigmentation had little significance in the genus, the species name should not describe colony colour. In my earlier papers I used the binomial Staphylococcus pyogenes for all coagulase-positive staphylococci, irrespective of their pigmentation, but when I assumed responsibilities to the International Committee on Bacteriological Nomenclature I was morally bound to use a name that was correct by the rules of the Bacteriological Code. Thus, before the publication of the paper on the classification of staphylococci by Shaw, Stitt and Cowan (1951), we went back to Rosenbach's original publication and found, to our chagrin, that he had never used the binomial Staphylococcus pyogenes; this combination could not be attributed to Rosenbach and its subsequent use by other authors only established it as a later synonym of Staphylococcus aureus.

In its first five editions the Bergey Manual correctly used Staphylococcus aureus, but in the sixth edition (ignoring its temporary use of Micrococcus) the form Staphylococcus pyogenes var. aureus was used. Unfortunately many workers accept too uncritically the nomenclature of the Manual (its editors do not claim to be infallible), and this combination has become popular. Recently Evans, Bradford, and Niven (1955) made the unfortunate statement, which needs correction, that "There seems to be a reasonable doubt as to whether the correct specific epithet should be aureus or pyogenes" and proposed that the latter epithet should be accepted.

Staphylococci were first described and named by Ogston (1882) but Rosenbach (1884) was the first to use Staphylococcus in a generic sense. These cocci were found in purulent

lesions and, as they had to be distinguished from the streptococci, the most obvious differences must have been in colony size and pigment. It was not unnatural that Rosenbach, wishing to indicate two features of the organism, should use the trinomial Staphylococcus pyogenes aureus. However, he realized that if he were to create scientific names in the sense of the botanists, he should use binomials, and the last few lines of page 18 of Mikro-Organismen bei den Wund-Infections-Krankheiten des Menschen show that he proposed to name the organisms Staphylococcus flavus or aureus and Staphylococcus albus. Reproductions of the relevant pages of Rosenbach's book provide all the evidence needed to refute the statement of Evans et al. (1955).

It is pertinent to ask whether Rosenbach really intended to use Staphylococcus flavus; as far as can be ascertained this is the only occasion on which he used the name, subsequently the golden-yellow colour was referred to by the epithet aureus or by one of the contractions of which he seemed so fond (see later). He used Staphylococcus pyogenes aureus and Staphylococcus pyogenes albus as cross-headings on pp. 19 and 21 respectively, but on p. 27 Staphylococcus aureus, as a cross-heading, was followed on the next line by "(mit oder ohne albus)."

Rosenbach was inconsistent and, like many surgeons, had the bad habit of using shortened forms of words, also, somewhat surprisingly in a German, he often omitted upper case (capital) letters. In his book neither italic type nor spacing of letters was used to indicate scientific names so that his abbreviated forms appeared as shown below:

"von staphylococcus pyog. aur."	p. 36, line 5
"staph. pyog. albus"	p. 36, line 21
"staph. pyog. aur.	p. 36, line 23
"strept. pyog."	p. 38, line 12
"staphyloc. p. aureus"	p. 39, line 3
"staph. alb."	p. 40, line 25
"Staphyl. aureus und albus"	p. 52, line 19
"Staphyl. aur."	p. 116, line 24

It might be argued that as Rosenbach used Streptococcus pyogenes (p. 22) he intended his trinomial for staphylococci to indicate varieties of a species Staphylococcus pyogenes, but even allowing for his inconsistencies and his love of abbreviation, the word "pyogenes" did not appear when he used binomials, either in full or in shortened form, except in his

reference to the streptococcal species (p. 38, "strept. pyog."). It is not likely that careless reading of Streptococcus pyogenes has been interpreted to mean that Rosenbach used Staphylococcus pyogenes and it certainly does not explain Hucker's use of the varietal forms in the 6th edition of the Bergey Manual, where Rosenbach's usage is faithfully recorded, except for the omission of a reference to the last two lines of p. 18, which read "Zur Bezeichnung der beiden Arten schlage ich vorläufig, nach der charakteristischen Farbe der Kultur die Namen Staphylococcus flavus oder aureus und Staphyl. albus vor." (Pages 18, 19, and 27 are reproduced in this number of the BULLETIN.

CONCLUSIONS

It seems that the persistent use of Staphylococcus pyogenes is another example of appropriateness being considered more important than the rules of nomenclature. Perhaps the reproduction of these pages from Rosenbach's book will convince those who abide by the rules of bacteriological nomenclature that the correct binomial is Staphylococcus aureus Rosenbach.

Gibson (1953) has asked the Judicial Commission to recognize Staphylococcus aureus as the type of the genus Staphylococcus, and the time seems ripe for an Official Opinion that will fix the name of this species. At the same time, a neotype strain should be designated; the strain S 11 R_4 (NCTC 8532), proposed by Cowan, Shaw and Williams (1954) has the support of Evans, Bradford, and Niven (1955) and should be considered in preference to the strain FDA 209 P proposed by Shaw, Stitt and Cowan (1951) and by Gibson (1953).

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I should like to express my thanks to J. F. Bergmann-Verlagsbuchhandlung, München, for allowing me to reproduce the relevant pages of Mikro-Organismen bei den Wund-Infections-Krankheiten des Menschen, to the Librarian of the Royal Society of Medicine for putting me in touch with Rosenbach's publishers, and to Mr. W. Clifford of the Central Public Health Laboratory, Colindale, for the photographs.

oder nach Ogston in wolken-, trauben- oder fischrogenförmigen Conglomeraten auftretenden Coccus bezeichnen. Ich will mich hier an Ogston's Bezeichnungen anschliessen und den Namen Staphylococcus adoptiren, freilich nicht für einen einzigen Pilz, sondern für eine Gruppe von vorläufig 2 Einzelarten. Diese beiden Arten verhalten sich in ihrem Wachsthum, ihrer mikroskopischen Form, in ihrer Gruppirung, in ihrer pathogenen Wirkung beim Thierversuch so analog, dass man sie nicht würde unterscheiden können, wenn sie sich nicht sofort in den Kulturen sehr auffällig durch ihre Farbe markirten. Die eine häufigste Art macht goldgelbe, undurchsichtige, die andere scheinend weisse, auch undurchsichtige Kulturen (Fig. I und IV). Diese Farbenunterschiede werden beibehalten in allen Generationen, mag der Nährboden in verschiedenster Weise wechseln: Gelatine mit Agar, mit Kartoffeln, Blutserum etc. mag die Serie der Kulturen durch Eiweiss, Fleisch mit und ohne Luft, mag sie vom lebenden Thier zurück zum todten, Nährboden führen. Im Eiter kommen diese beiden Arten nicht selten zusammen vor, wahrscheinlich noch häufiger, als ich im Anfang dieser Untersuchungen glaubte. Es haben nämlich gleichaltrige Kulturen auf Agar die Eigenschaft, da, wo sie sich berühren, so diffus in einander zu wachsen, dass man eine gelbe Reinzucht vor sich zu haben wähnt, während in der That eine Mischzucht vorhanden ist. So sah ich einige Male beim Aufstreichen eines coccenarmen Eiters weisse und goldgelbe Pünktchen, im bunten Bilde gemischt aufgehen; wo die weissen Pünktchen allein lagen, blieben sie weiss, sobald sie einen gelben Punkt berührten, diffundirten beide Kulturen zu einem gelben Flecke. Zur Bezeichnung der beiden Arten schlage ich vorläufig, bis die Botaniker diese Wesen mit passenden Benennungen in das allgemeine System eingereiht haben werden, nach der charakteristischen Farbe der Kultur die Namen Staphylococcus flavus oder aureus und Staphyl. albus vor. Freilich wird

man zum Unterschied von anderen gelben Coccenarten noch angeben müssen, dass sie aus (menschlichem) Eiter erhalten wurden. Betrachten wir also zuerst den

1. Staphylococcus pyogenes aureus.

Ich habe diesen, wenn ich alle meine Culturen, auch die im Folgenden nicht speciell erwähnten zusammennehme, wohl am häufigsten angetroffen. Macht man eine Aussaat dieses Mikrobions, sei es des im Eiter enthaltenen oder des schon gezüchteten in Form eines Impfstriches auf F. P. A., so entsteht bei 30—37° Cels. Brütungstemp. schon nach 24 Stunden, selbst früher ein schwach opaker Strich, welcher sehr bald deutlicher wird und dann aussieht, wie wenn er mit Anfangs weisslich-gelber, später orange-gelber Oelfarbe aufgetragen wäre. Die Kultur wächst dann in die Breite, indem sie rundliche Facetten bildet bis zu 3 auch 4 mm und nimmt einen noch immer dunkleren orangefarbenen Ton an (Fig. I, II, III), dann hört sie auf, sich auszudehnen. Sie wächst spontan nicht in die Tiefe. In der Kälte wächst sie langsamer. Im Impfstich wächst sie ebenfalls gut und bildet eine undurchsichtige gelbe, stellenweise unregelmässige, klumpige Säule. Auf F. P. G. verflüssigen sich die Impfstriche sehr bald, und später der übrige Theil der Gelatine (ohne dass der Coccus dieselbe vorher durchwächst). Die Kultur fällt dann zu Boden und wird allmählich zu einem dunkel-orangefarbigen Satz. Auf erstarrtem Blutserum entstehen rasch wachsende Kulturen, Anfangs nur schwach gelb, später dunkler. Auf Kartoffeln ebenfalls gutes Wachsthum. An der Luft trocknet die Kultur mit der Zeit ein, verliert die Farbe und wird schwieriger zu übertragen, ohne dass sie jedoch nach fast Jahresfrist (in einem Falle) sich gänzlich abgestorben zeigte. Ohne Luft hält sie sich sehr lange. Ich komme darauf bei den Beobachtungen über Osteomyelitis zurück. Das Mikrobion stellt sich mikroskopisch als ein sehr kleiner Coccus dar

zu Tage; ich kann daher specieller auf dieselben erst nach Mittheilung des gesammten klinischen Materials, der Abscesse, Empyeme und der schwereren Phlegmone eingehen.

III. Klinische Mittheilung der Fälle acuter Abscesse nach den in ihnen gefundenen Mikrobien geordnet.

Bei den 26 Fällen geschlossener Abscesse, von denen ich die Krankengeschichte besitze, sind anfangs die Culturen auf F. P. G. gemacht, und die meisten der folgenden ohne speciell darauf gerichtete Differenzirungs-Untersuchungen, nach Koch's Methode geblieben, so dass ich nicht mit Sicherheit behaupten kann, ob nicht ab und zu beide Staphylococcusformen vorhanden waren, während Streptococcus in den Culturen mikroskopisch kaum zu übersehen sein dürfte. Es fand sich

A. Staphylococcus aureus

(mit oder ohne albus) in folgenden Abscessformen:

1. Abscess der Kopfdecken bei 3jährigem Kinde unter Kopfeczäm entstanden. Baldige Heilung.
2. Präpatellarabscess bei 20jährigem Dienstmädchen mit promptem Heilungsverlauf.
3. Submentaler Abscess mit dickem rahmigen Eiter bei Kind, seit 8 Tagen entstanden. Baldige Heilung.
4. Abscess nach Phlegmone der submaxillaren Drüsen, seit 8 Tagen entstanden bei einem 4jährigen Mädchen.
5. Phlegmonöser, tiefer, subfascialer Abscess am unteren Ende des Radius bei 3jähr. Kind, ohne bekannte Ursache entstanden. Ursprüngliche Osteomyelitis-Diagnose: nicht bestätigt, sondern rasch heilender Weichtheils-Abscess.
6. Abscess der submentalen Drüsen nach Ausschlag um Mund und Kinn.
7. Abscess am oberen Umfang der mamma, seit 14 Tagen entstanden bei 17jährigem Mädchen.
8. Abscedirung der Cruraldrüsen, nach einer vor 4 Wochen entstandenen Schrunde am malleol. int. bei einem 15jährigen Knaben, rasche Heilung.
9. Präpatellarphlegmone bei 18jährigem Dienstmädchen.

Page 27 of: Rosenbach, F.J. Mikro-Organismen bei den Wund-Infections-Krankheiten des Menschen.

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BIOCHEMICAL TESTS FOR BACTERIAL CHARACTERIZATION. 7. GLUCONATE TEST.

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For characterizing Pseudomonas aeruginosa, Haynes (1951) described a medium in which the conversion of potassium gluconate to potassium 2-ketogluconate was detected by the appearance of reducing substances. Shaw and Clarke (1955) used a modification of this medium in their study of Providence cultures and developed from the test a micromethod using heavy suspensions of living organisms. These tests were said to be useful in taxonomic work on the klebsiella group, but in my hands the microtest did not always give the same result as the culture growing on the gluconate medium. This paper records experiments made to find the optimal conditions for the microtest, and also describes a microtest suitable for routine use in taxonomic work.

Methods

Media. A simple Lemco agar (LA) medium was made up as follows: Lemco, 10 g.; peptone, 10 g.; NaCl, 5 g.; water, 1 litre; pH adjusted to 7.6; sterilized at 10 lb. pressure for 10 minutes. To this base (LA) might be added 1 per cent glucose (GA) or other carbohydrate.

Suspensions. Strains were grown on LA and on GA at 30° C. for 18-24 hours; growth was washed off with physiological saline, spun, and the deposit resuspended in distilled water. Suspensions made from growth on Lemco agar (LA) are identified in this paper by the designation LAS, those made from glucose agar (GA) by GAS. Some klebsiella strains were so mucoid that the cells would not pack on centrifugation; in these instances as much as possible of the supernatant was removed and the cells were resuspended in the remaining syrupy fluid. To compensate for this inability to concentrate, these lighter suspensions were added in greater volume in the microtests. For strains that packed well on centrifugation, 0.75 ml. water was added to the deposit from one slope in a 6 x 1/2 inch tube.

Solutions. Gluconate solutions were made either by dissolving potassium gluconate crystals or by adjusting a 1 per cent gluconic acid solution to the required pH value by adding normal potassium hydroxide (referred to as 1 per cent gluconate solutions).

Buffers. McIlvaine's buffer solutions were used in most experiments on the gluconate test. Sørensen's phosphate buffers were used in other tests (such as that for acetoin) made at the same time.

Gluconate test. Conditions of the test are shown at the head of each table. After incubation of the gluconate and suspension mixtures 0.1 ml. Benedict's qualitative reagent was added to each tube, and tubes were placed in a water-bath at 100°C. for 5 minutes. A positive test showed reduction of the reagent.

Experimental

The mucoid nature of many klebsiella organisms prevented their complete sedimentation on spinning; and it was necessary to find whether variation in suspension concentration materially affected the result of the gluconate test. Six nonmucoid strains were grown on LA and on GA, and the centrifuged cells were resuspended in distilled water. Twofold dilutions of each suspension were made in water, and each tested in duplicate by the gluconate microtest described by Shaw and Clarke (1955). The results of this experiment (Table 1) showed that, in general, the test was not particularly sensitive to changes in suspension concentration. Strains No. 2794 and No. 5055 gave unexpected results in that the weaker suspensions from glucose agar (GA) were positive, while the more concentrated suspensions were negative.

The medium on which the strain had been grown seemed to have a variable effect on this test; in this experiment suspensions of two strains were positive when grown on LA and only feebly positive from GA. In later experiments with a slightly modified method, GAS of these strains gave positive results. Three strains, NCTC 204, 3263, and 5056, were positive only in GAS. This association of a positive gluconate test with growth on GA was analogous to the VP microtest in which most VP-positive strains are negative when tested as suspensions from LA. Clarke and Cowan (1952) showed that

Table 1. Effect of (a) suspension concentration and (b) growth medium on the gluconate microtest.

(1% K. gluconate, 0.04 ml.; 0.025 M-phosphate buffer, pH 6.8, 0.04 ml.; suspension, 0.04 ml.)

Strain No.	Suspension	Undiluted	Suspension dilution 1/2	1/4	1/8
204	LAS	-	-	-	-
	GAS	+	+	+	+
2794	LAS	+	+	+	+
	GAS	-	-	-	+
3263	LAS	-	-	-	-
	GAS	+	+	+	±
5054	LAS	-	-	-	-
	GAS	-	-	-	-
5055	LAS	+	+	+	+
	GAS	-	-	±	±
5056	LAS	-	-	-	-
	GAS	+	+	+	+

LAS = suspension made from growth on Lemco agar.
GAS = suspension made from growth on glucose agar.

this was not a glucose adaptation, but an effect of reduction of pH value due to growth in a glucose-containing medium.

Experiments were made to find out whether the pH effect was primarily concerned with the reaction at which the test was made, or with the reaction of the medium on which the culture had been grown. 1 per cent gluconic acid solution was adjusted with N-NaOH to pH values of 4, 5, 6, and 7, and these were used with McIlvaine's buffer solutions of corresponding pH value. Gluconate-suspension mixtures were incubated at 37°C. for varying times. Benedict's reagent was then added and the mixtures were boiled.

The results of this (Table 2) and similar experiments showed that for most strains the optimal pH value for the test was 4, but for a few strains (see Table 4) the optimum was 6. The effect of the medium was found by adjusting the pH value; agar could not be used throughout the range as it failed to solidify at the lower pH values.

Table 2. Effect of (a) pH value and (b) duration of reaction on the gluconate microtest.

(1% gluconate solutions, 0.06 ml.; buffer, 0.06 ml.; suspensions, 0.04 ml. Test organism, NCTC 9132)

Suspension	Reaction time (hours)	pH value of gluconate solution 4	5	6	7
LAS	1	-	-	-	-
GAS	1	+	±	-	-
LAS	2	-	-	-	-
GAS	2	++	+	±	-
LAS	4	+	-	-	-
GAS	4	++	+	±	-
LAS	22	++	-	-	-
GAS	22	+	+	+	-

Table 3. Effect of growth medium on the gluconate microtest.

(Strain 9132 grown on LA and GA of pH 7.6 and on acid broth (AB) at pH 5. Gluconate solution at pH 4.)

Time of test (hours)	Suspensions LAS	GAS	ABS
1	-	+	++
2.5	-	++	++
5	±	++	++

LAS = suspension from growth on LA at pH 7.6.
GAS = suspension from growth on GA initially at pH 7.6.
ABS = suspension from growth on Lemco broth initially at pH 5.

Table 4. Effects of variation in pH value of (a) medium in which cultures were grown and (b) gluconate solutions in the test.

(McIlvaine buffer solutions were of the same pH values as the gluconate solutions. Tests were made after incubation for 6 hours.)

Strain No.	Medium	pH	pH of gluconate solution 4	5	6	7
9162	LB	5	+	+	-	-
	LB	6	+	+	-	-
	LB	7	+	±	±	-
	GB	7	+	±	±	±
8813	LB	5	+	++	++	+
	LB	6	+	++	+	-
	LB	7	+	++	+	-
	GB	7	+	+	+	-
8168	LB	5	-	-	-	-
	LB	6	-	-	-	-
	LB	7	-	-	-	-
	GB	7	-	-	+	+
9665	LB	5	-	-	+	-
	LB	6	-	-	+	-
	LB	7	-	-	+	-
	GB	7	+	+	+	+

LB = Lemco broth. GB = Lemco broth + 1 per cent glucose.

Cultures were grown in Lemco broth at pH 5 and the tubes were placed at an acute angle in the incubator so that there was a large air surface. The same strain was grown on LA (pH 7.6) and on GA (pH 7.6). Table 3 shows that growth in an acid medium had the same effect as growth in a glucose medium, and from this it can be concluded that the gluconate test is not dependent on adaptation to glucose. However, the effect of variation of pH value both of the medium and of the test is complex, as is shown by the results presented in Table 4.

In each test the suspension was mixed with gluconate solution and buffer and the mixture kept in a water-bath at 37°C for a definite time before Benedict's reagent was added. Table 5 shows the effect of varying the duration of the test; most strains resembled No. 9162 in giving a stronger reaction (i.e. showing more complete reduction of the Benedict's reagent) after 24 hours, but as this type of reaction was not invariable it was necessary to carry out the test on a representative collection of strains.

Table 5. Effect of time factor on the gluconate test.

(1% gluconate solution, pH 4, 0.06 ml.; McIlvaine buffer, pH 4, 0.06 ml.; suspension, 0.04 ml.)

Strain No.	Time of test (hours)	Suspension			
		LBS 5	LBS 6	LBS 7	GBS 7
9162	2	+	+	-	+
	4	+	+	+	+
	6	+	+	+	+
	24	++	++	++	++
9665	4	-	-	-	-
	6	-	-	-	+
	24	-	-	-	-

LBS = suspension from growth in Lemco broth; figure represents pH value of the medium before inoculation.
GBS 7 = suspension from growth in Lemco broth + 1 per cent glucose at pH 7.

A series of 109 strains (92 of these being Aerobacter or Klebsiella spp.) was tested, using both LAS and GAS suspensions, gluconate solutions of pH 4 and of pH 6, and incubating the mixtures for 4 and 24 hours at 37°C. The results of this trial showed that 62 strains were gluconate-positive in one or more combinations of pH and time, and that GAS tested after 4 hours at pH 4 gave the greater number of positives (Table 6). Further analysis (Table 7) showed that LAS suspensions were not positive when GAS were negative, and that some strains were only positive under certain pH-time combinations.

Table 6. Results of gluconate microtests on 92 strains of Aerobacter and Klebsiella cultures; tests made at different pH values and after varying intervals.

pH of test	Reaction time (hours)	No. positive LAS	No. positive GAS
4	4	29	55
4	24	38	52
6	4	3	22
6	24	3	16

LAS = suspensions made from growth on Lemco agar.
GAS = suspensions made from growth on glucose agar.

Table 7. Analysis of positive gluconate results.

Total number positive	62 strains
Positive only at 4 hours	5 "
Positive only at 24 hours	5 "
Positive only with LAS	0 "
Positive only with GAS	23 "
Positive only at pH 4	33 "
Positive only at pH 6	6 "
Positive only at pH 4 and 4 hours	1 "
Positive only at pH 6 and 4 hours	1 "
Positive only at pH 6 and 24 hours	5 "

It is clear from Table 7 that the gluconate microtest cannot be a single tube test; it must be a series of tests, exploiting the combinations of pH value and duration of incubation found most useful. It is suggested, therefore, that the gluconate microtest should be performed in duplicate at pH 4 and 6, and tests made after incubation for 4 and 24 hours. Two gluconate solutions are required; 1 per cent gluconic acid is adjusted with N-NaOH or N-KOH to pH 4 and pH 6. The test mixture consists of gluconate solution, 0.06 ml.; McIlvaine buffer solution of the same pH value, 0.06 ml.; suspension (GAS), 0.04 ml.; control tubes of water, 0.06 ml.;

and GAS suspension, 0.04 ml. are included. The mixtures are incubated at 37°C and after 4 hours two sets of tubes (one test and one control at pH 4 and another pair at pH 6) are withdrawn and 0.1 ml. Benedict's reagent is added to each tube. All four tubes are placed in a water-bath at 100°C for 5 minutes. Reduction of the Benedict's reagent indicates a positive gluconate test. The other sets of tubes are tested by adding the Benedict's reagent after incubation at 37°C for 24 hours.

DISCUSSION

The gluconate microtest, carried out at two pH levels and after different reaction times, gave results which were easy to read and were readily reproducible. The microtest, however, did not give exactly the same results as the test with growing cultures used by Shaw and Clarke (1955). The same strains were examined by Miss C. Shaw, using cultures growing in a gluconate medium, and by me using the microtest described here. An unexpected discrepancy was found in strains which, in the microtest, were positive only with suspensions from growth on glucose agar (GAS); these strains were negative when grown in the gluconate medium. It remains to be seen whether the strains behaving in this way fall into one group, and whether different results in the macro- and microtests have any taxonomic significance.

SUMMARY

1. A microtest was developed to detect the ability of bacterial suspensions to convert potassium gluconate to potassium 2-ketogluconate.

2. Suspensions prepared from growth on glucose agar, or Lemco broth at pH 5, gave more positive results than suspensions from Lemco agar or broth at pH 7-7.6.

3. Most suspensions were more active at pH 4 than at pH 6, but there were enough exceptions to justify carrying out the test at both pH values.

4. Tests made after 24 hours of incubation gave stronger

reactions than those after 4 hours; a few strains were positive only when tested after 4 hours.

5. The microtest proposed should be made with (a) suspensions from growth on glucose agar, (b) at both pH 4 and 6 and (c) after 4 and 24 hours of incubation at 37°C.

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TWO NEW SALMONELLA TYPES: S. SEATTLE AND S. PORTLAND

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Salmonella portland (culture 4484-54) was isolated in the laboratories of the Oregon State Department of Health and forwarded to the writers by Dr. William Levin as a group D Salmonella serotype with a request for specific identification. The organism was found in abdominal fluid obtained from a 60-year-old female patient by paracentesis. Two weeks prior to hospital admission the patient had been affected with chills, fever, anorexia, chest pains, and jaundice. After treatment with various antibiotics the temperature subsided to 102°F. Nothing was learned concerning the subsequent course of the illness.

S. portland produced hydrogen sulfide, failed to form indol, to hydrolyze urea, or to liquefy gelatin, was methyl red positive and Voges-Proskauer negative. The organism grew readily on Simmons' citrate agar but failed to grow on Moeller's KCN medium. The organism was motile, reduced nitrates, and acidified Jordan's tartrate agar. Glucose, xylose, arabinose, rhamnose, trehalose, mannitol, and sorbitol were fermented promptly with the production of gas. Unlike most salmonellae, S. portland produced acid and gas from raffinose after 24 hours incubation. Lactose, sucrose, salicin, inositol, dulcitol, and adonitol were not fermented.

The culture was agglutinated to the titre of Salmonella gallinarum O serum (9, 12) and in absorption tests removed all agglutinins from the serum. The H antigens were diphasic. Phase 1 was agglutinated to the titre of Salmonella illinois, phase 1 (z_{10}) serum. In absorption tests S. portland reduced the titre of the serum from 10,000 to 500 and left a residue of agglutinins for phase 1 of Salmonella eschweiler (6, 7 : z_{10} - 1, 6), Salmonella tel hashomer (11 : z_{10}-e, n, x), and an unnamed Salmonella type having the formula 11 : z_{10}-1, 2.

Phase 2 of S. portland was agglutinated by serums derived from all the nonspecific phases of the genus. In tests with single factor serums, agglutination occurred in absorbed

serum for factor 5 but not in serums for factors 2, 6, or 7, The organism reacted to the titre of serum for phase 2 of Salmonella thompson (1, 5) and in absorption tests reduced the titre of the serum from 20, 000 to 1, 000. All agglutinins for phase 2 of Salmonella panama (9, 12 : 1, v-1, 5) were removed from the serum. The antigenic formula of S. portland is 9, 12 : z_{10}-1, 5.

Salmonella seattle (culture 2775-54) was isolated in the laboratories of Seattle-King County Department of Health from the feces of a 6 weeks old child affected with severe diarrhea and was forwarded for identification by Miss M. Mulhern. The organism was found repeatedly in the stools of the infant. The child, as well as two siblings and the mother, developed gastrointestinal symptoms in Khartoum before leaving for the United States. No pathogenic forms were recognized in the stools of the siblings but Shigella flexneri 4 was isolated from the mother.

The biochemical properties of S. seattle were the same as those of S. portland except that the former fermented dulcitol promptly and failed to ferment raffinose. Upon serological examination it was found that S. seattle was agglutinated to the titre of antiserum of Salmonella O antigen 28. This serum, which was prepared from a monophasic Salmonella culture having the antigenic formula 28 : y, retained a pronounced residue of agglutinins for the homologous strain after absorption with S. seattle. However, there was a complete removal of agglutinins for three unnamed Salmonella types possessing O antigen 28.

The H antigens of S. seattle were diphasic and phase 1 agglutinated to the titre of, and removed all H agglutinins from, serum derived from Salmonella paratyphi A (a). Phase 2 was agglutinated actively by serums derived from antigens e, n, x and e, n, z_{15}. It reacted with absorbed serum for factor x but not with serum for factor z_{15}. In absorption tests the organism removed all H agglutinins from serum derived from Salmonella abortus-equi (e, n, x). The antigenic formula of S. seattle is 28 : a-e, n, x.

SUMMARY

Two new Salmonella serotypes isolated from infections in man are described. S. portland represented by the antigenic formula 9, 12:z_{10}-1, 5; S. seattle by the formula 28:a-e, n, x.

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TAXONOMIC PROBLEMS RELATING TO THE IDENTIFICATION OF SPECIES WITHIN THE GENUS PSEUDOMONAS

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The 6th ed. of Bergey's Manual (1948) lists 261 names of species in the genus Pseudomonas, of which 148 are fully accepted and described in detail, 15 are believed better placed in the genus Xanthomonas, and 98 are listed in the genus as names of uncertain position. The genus is the largest in the 1948 edition of Bergey's Manual.

New species names are constantly being added to the genus Pseudomonas, frequently on the basis of some variable minor characteristics or solely on the basis of flagellation regardless of physiological characteristics. In many instances the species characteristics now listed are so vague and inadequate that laboratory reidentification of a culture that had lost its label would be impossible. This point is emphasized by the fact that, with few exceptions, investigators studying the genus (primarily the species P. aeruginosa and P. fluorescens) do not agree on the major characteristics of the group. These controversies include such basic and fundamental differences as typical colonial morphology, pigmentation, serological relationships, biochemical or physiological characteristics, odor, optimum temperature of growth, and even the ease, or difficulty, of overall identification of the genus. (1, 2, 5, 6, 7, 9, 10, 11, 13, 15, 16, 17)*

The type species of the genus, P. aeruginosa, can be isolated not only from feces, urine, skin burns, abscesses, chronic lung diseases, etc., but also from soil, water, and even plants. Because of the ubiquitous distribution of this and other species in the genus, when studying their characteristics, one must recognize and consider carefully their adaptability and dissociation. It is well known, for example, that cultures of P. aeruginosa frequently lose their

*For a complete list of references, see Gaby, 1946 (6); Munoz et al., 1949 (13); and Haynes, 1951 (9).

ability to produce pigment and that freshly isolated strains may occasionally be nonpigmented. Under these conditions P. aeruginosa may be indistinguishable from numerous other Pseudomonas species. Studies carried out in this laboratory show that similarities exist among many of the species of the genus and that differences in their biochemical characteristics can be due to variations which commonly occur within this group of organisms.

Methods and Materials

Special attention has been given to all cultures isolated from clinical specimens and identified as P. aeruginosa in this diagnostic laboratory. Over 300 such cultures have been examined to date. Identification was based on the characteristics previously described by Gaby and Free (7). In addition, the physiological and morphological characteristics of 12 species of Pseudomonas, other than P. aeruginosa, obtained from the American Type Culture Collection, were compared with the clinical cultures and stock cultures of P. aeruginosa. All cultures were grown on heart infusion agar or broth. Biochemical characteristics were determined by standard techniques. All carbohydrate media were prepared in a peptone base broth. Cultures were incubated at 37°C, and room temperature (approx. 24° - 25°C) for appropriate periods of time ranging up to three weeks before the final readings in litmus milk, gelatin, and carbohydrate media were recorded.

Results and Discussion

The biochemical characteristics of these species and strains are given in the table. Only the results obtained with five of the clinical cultures are shown to represent the degree of variation obtained in biochemical characteristics. Although the reactions observed are relatively constant, variations do occur. The two most variable reactions noted were those in litmus milk and carbohydrate media. It should be noted that the reactions listed here do not necessarily agree with those found in Bergey's Manual (1948). All of the species fell within the limits of biochemical variations found for strains of P. aeruginosa, with the possible exception of the last four species listed in the table. A sharp distinction

between several of the species, however, is evident in their optimum growth temperature range. Those species that grew well at 37°C, also grew well at room temperature (24°-25°C). However, 7 of the species did not grow as well at 37° as they did at room temperature.

It is evident from the data presented that the problem of species differentiation has two aspects. First, and perhaps the more important, is the similarity of P. aeruginosa to several other species of Pseudomonas; e.g., P. chlororaphis and P. putida were indistinguishable from P. aeruginosa on the basis of biochemical and growth characteristics, whereas, P. mucidolens, P. fluorescens, P. putrefaciens, P. graveolens, P. ovalis, P. mildenbergii, and P. fragi differed primarily from P. aeruginosa in their optimum growth temperature. Second, there is the problem of distinguishing among species of Pseudomonas; e.g., P. mildenbergii was found to be indistinguishable from P. ovalis and P. fluorescens was indistinguishable from P. putrefaciens.

On the basis of the fermentative metabolism of carbohydrates by P. hydrophila and other similar species, their inclusion in the genus Pseudomonas may be questioned. Hugh and Leifson (1953) pointed out the basic differences between the fermentative and oxidative metabolism of carbohydrates by Gram-negative bacilli and discussed the taxonomic significance of these reactions. On the basis of the results obtained in this laboratory with the various species and strains of Pseudomonas as well as the routine examinations of other Gram-negative bacilli isolated in the clinical laboratory, such as the paracolons, Proteus, Salmonella, and Shigella, it was concluded that the following four biochemical characteristics should tend to exclude an organism from the genus: 1. A fermentative carbohydrate metabolism; 2, acid production from sucrose; 3, acid production from lactose; and 4, production of acid curd in litmus milk.

The question naturally arises as to what are the characters of the type species P. aeruginosa, and what procedures should a laboratory follow in making a positive identification. Should such metabolic activities as the nonphosphorylated oxidation of glucose by way of gluconic and 2-ketogluconic acids by P. aeruginosa as described by Campbell and Norris (3) be considered a basic characteristic? Haynes (9) considers this characteristic along with maximum growth temperature and slime formation as sufficient evidence for the

identification of P. aeruginosa. However, it has been demonstrated in this laboratory that several strains of Proteus and paracolons possess one or more of these characteristics.

It should perhaps be reemphasized at this time that the pigmented strains are easily identified. The nonpigmented strains offer a real problem of correct or satisfactory identification if one adheres strictly to the keys and descriptions in Bergey's Manual. For example, where should one place a Gram-negative, nonpigmented, motile bacillus, producing only acid in glucose and sucrose, lactose negative, urea and gelatin negative, isolated from urine, or water? The taxonomist immediately points out that in this so-called hypothetical question the presence or absence of polar flagella is not mentioned. However, flagella staining is not routine in most laboratories and would not usually be regarded as necessary in this case as most clinical laboratories would regard this organism as a paracolon rather than as a Pseudomonas on the basis of acid production from sucrose. However, a lack of pigment alone does not rule out allocation to the genus Pseudomonas. Numerous examples could be cited to illustrate possible conflicting views as to the identity of many such borderline microorganisms. Studies are currently under way in this laboratory to determine the extent of variation and dissociation of P. aeruginosa. Until this has been satisfactorily determined one cannot expect to get agreement as to the circumscription of the type species. There is no doubt, however, as the data presented indicate, that these boundaries will incorporate many of the species now recognized. These findings are adequately substantiated in the literature, as Mehta and Berridge (12) have reported that not only did a close relationship exist between certain species of the genus Pseudomonas, but also that P. aeruginosa and P. marginale were identical in morphological and cultural characteristics and in pathogenicity on lettuce. Paine and Branfoot (14) found that P. aptata is identical with, and probably a strain of, P. aeruginosa. Elrod and Braun (4) concluded P. polycolor to be indistinguishable from cultures of P. aeruginosa. These authors also confirmed the results of Mehta and Berridge as to the identity of P. aeruginosa and P. marginale. There are many such examples in the literature, but for some reason they have been, with few exceptions, ignored in Bergey's Manual.

BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY

BIOCHEMICAL REACTIONS

Species	Source	Optimum temperature	Nitrate reduction	Gelatin liquefaction	Litmus milk	Indole production	Glucose	Sucrose	Xylose	Lactose
P. aeruginosa R*	No.51 stock	24°-37°	+	+	P	-	A	-	A	-
P. aeruginosa R*	No.52 stock	24°-37°	+	+	P	-	A	-	A	-
P. aeruginosa	Clinical lab.	24°-37°	+	+	P	-	A	-	A	-
P. chlororaphis	ATCC	24°-37°	+	+	P	-	A	-	A	-
P. aeruginosa	Clinical lab.	24°-37°	+	+	P	-	A	-	-	-
P. aeruginosa	Clinical lab.	24°-37°	+	+	ALK.	-	A	-	A	-
P. mucidolens	ATCC	24°**	+	+	ALK.	-	A	-	A	-
P. aeruginosa S*	No.52 stock	24°-37°	+	+	P	-	-	-	-	-
P. fluorescens	ATCC	24°**	+	+	P	-	-	-	-	-
P. putrefaciens	ATCC	24°**	+	+	P	-	-	-	-	-
P. aeruginosa S*	No.53 stock	24°-37°	-	+	P	-	A	-	A	-
P. aeruginosa S*	No.51 stock	24°-37°	-	-	ALK.	-	A	-	A	-
P. putida	ATCC	24°-37°	-	-	ALK.	-	A	-	A	-
P. aeruginosa	Clinical lab.	24°-37°	-	-	ALK.	-	A	-	A	-
P. ovalis R*	ATCC	24°**	-	-	ALK.	-	A	-	A	-
P. mildenbergii	ATCC	24°**	-	-	ALK.	-	A	-	A	-
P. graveolens	ATCC	24°**	-	-	-	-	A	-	A	-
P. aeruginosa	Clinical lab.	24°-37°	-	-	-	-	A	-	A	-
P. fragi	ATCC	24°**	-	-	-	-	A	-	-	-
P. pavonacea	ATCC	24°-37°	-	-	ALK.	-	-	-	-	-
P. oleovorans	ATCC	24°-37°	-	-	ALK	-	-	-	-	-
P. ovalis S*	ATCC	24°**	-	-	ALK.	-	-	-	-	-
P. hydrophila	ATCC	24°-37°	+	+	P	+	AG	AG	AG	-

A = Acid. AG = Acid and gas. P = Peptonization. Alk = Alkaline. + = Positive reaction. - = No reaction.

* R = Rough, S = Smooth. From those species containing easily recognized R and S colonies.

** All cultures grew at 37°C. On repeated transfers at 37°, growth improved slightly but did not approach that observed at the optimum temperature.

AIthough no 'type culture' of the species P. aeruginosa has thus far been designated, it is possible to delimit the type species more accurately than is done in Bergey's Manual. The genotype P. aeruginosa is comprised of a highly dissociable group of Gram-negative, non-encapsulated rods. Cells are monotrichous, lopothrichous, or rarely, nonmotile. Pigments may or may not be produced. If pigments are formed they may be green (pyocyanin), brown (pyorubin), or fluorescent. The ability to produce pigments, especially pyocyanin, is frequently lost on repeated subculturing. Indeed, many freshly isolated cultures identified as P. aeruginosa on the basis of other characteristics do not produce pigment. Glucose and occasionally xylose are usually the only carbohydrates fermented with the production of acid in a peptone base medium, whereas in a synthetic medium acid may be produced from glucose, xylose, arabinose, galactose, glycerol, and mannose. Nitrates are usually reduced to nitrites. Gelatin is usually liquefied, especially in freshly isolated cultures. Indole formation is variable. Litmus milk usually becomes alkaline and peptonized. Fats and hydrocarbons may or may not be attacked. A pellicle is usually produced in liquid culture media. The colonial morphology is variable but can be classified into three basic types. 1. Large, spreading, with generally smooth, convex, translucent centers; effuse, flat, wavy transparent periphery; and irregular, lobulated edges. 2. Round, convex, translucent, finely granular or mucoid with entire edges. 3. Round or slightly irregular, slightly raised, umbilicate or umbonate, finely or coarsely granular, simulating a rough colony. Representatives of this species are commonly found in water, soil, vegetable matter, and are opportunists, frequently causing secondary infections in man.

Summary

A study of clinical and stock cultures of P. aeruginosa and 12 other species of Pseudomonas obtained from the American Type Culture Collection indicated that many of these cultures are indistinguishable from one another and from P. aeruginosa when their morphological and biochemical characteristics are determined by standard procedures. While there are, no doubt, several distinct species of Pseudomonas, it is evident that there have been placed in the

genus many ill-defined and illegitimate species. No type culture of Pseudomonas aeruginosa has thus far been designated. It is therefore as yet impossible accurately to describe it. An amended description of the type species, however, is given which is more accurate than that given in Bergey's Manual.

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A NOTE ON THE TAXONOMY OF LACTOBACILLUS BIFIDUS

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During recent years increasing attention has been drawn to Lactobacillus bifidus, especially in investigations of its nutrition. It seems, however, that some confusion still occurs as to the taxonomy of this bacterium.

According to Weiss and Rettger (1), L. bifidus is characterized by its strict anaerobic behaviour in freshly isolated cultures, and its very variable cell shape. Weiss and Rettger (2) later called attention to a possible subspecies, L. parabifidus, which they considered more permanently anaerobic than L. bifidus, and capable of fermenting pentoses. These considerations of Weiss and Rettger have been accepted in the current edition of Bergey's Manual (3).

Later authors have paid attention to the "straight rod variants" of L. bifidus, which may develop from branched cultures. The change in morphology is accompanied by development of the ability to grow aerobically. Norris et al. (4) have concluded that an inclusion of such aerobic straight rod cultures under the conception "L. bifidus" must be incorrect. Accordingly, they proposed that the species pattern should be limited to the obligate anaerobic branched type only, and that the name L. parabifidus should be employed for the aerobic (or microaerophilic), frequently straight rod cultures. Norris et al. considered the "straight rod type" to be a variant of the anaerobic branched type. Hence the proposal of these authors was exactly the reverse of the considerations of Weiss and Rettger (1,2).

From the definition of the variant conception outlined by Smith, Gordon, and Clark (5) ("A variant is an organism showing some variation in some character from the pattern of the stable parent species") it follows that the proposal of Norris et al. (4) is obscure. L. bifidus (Norris et al.) is distinguished from L. parabifidus (Norris et al.) on the basis of differences in morphology and in sensitivity to oxygen. So far as these characteristics are concerned L. bifidus provides no stable species pattern. L. parabifidus, again, may

not be a variant of its variable parent culture (L. bifidus). For the same reason L. parabifidus (Weiss and Rettger) can hardly be considered a subspecies of L. bifidus (Weiss and Rettger).

Recently the present author (6) cited evidence to show that primary cultures may possess the characters of L. parabifidus (Weiss and Rettger) and L. bifidus (Norris et al.). On subcultivation in vitro, the cultures showed a gradual change in their characters, especially cell morphology. The change was from a slightly branched anaerobic type to a microaerophilic type showing manifold branchings and mycelial filaments. Ultimately, straight rods developed from the filaments. The "straight rod" cultures were often aerobic. Both the latter types--highly branched forms or regular straight rods--most closely correspond to L. bifidus (Weiss and Rettger) and L. parabifidus (Norris et al.).

These findings show that the polymorphic appearance and the variation in sensitivity to oxygen may be distinctive characters of L. bifidus, and accordingly, that aerobic or microaerophilic types showing variation in morphology cannot be considered variants or subspecies of the ordinary anaerobic and branched type of L. bifidus.

Recently Rose and György (7) have reported that there occur two nutritionally different types of L. bifidus: a type which requires a specific factor present in human milk as an essential nutrient, and another type which does not require this factor for growth. These authors consider it probable that the former type, which they have named L. bifidus var. pennsylvanicus, is the parent organism, which may mutate into the latter, non-milk-requiring "regular" form of L. bifidus. Accordingly, the characteristic property of L. bifidus var. pennsylvanicus can be lost by variation, and then strains of L. bifidus var. pennsylvanicus may not vary from typical L. bifidus strains. It has not been reported that ordinary L. bifidus strains change, spontaneously or on training, into the milk-requiring type, and thus it can be concluded that L. bifidus in this respect provides a "stable parent species". L. bifidus var. pennsylvanicus, therefore can be considered a true variant of L. bifidus.

On the basis of the facts presented above it can be concluded that Lactobacillus bifidus (Tissier) Holland (L. bifidus Weiss and Rettger; cf. Bergey's Manual (3)) is to be considered as species pattern. The description given in the

1948 Bergey's Manual, however, ought to be amended in certain paragraphs, especially the characteristic variation in morphology and in sensitivity to oxygen must be emphasized. It would then read:

- - -

Small, slender rods....., very variable in appearance. Slightly branched and club forms are characteristic of freshly isolated cultures. On subcultivation highly branched filaments or regular straight rods may develop.

- - -

Strict anaerobe in primary culture becoming microaerophilic. Straight rod cultures often show surface growth in stabs.

- - -

Distinctive characters: Bifurcations and club-shaped forms, particularly in primary culture. On subcultivation highly branched filaments may develop, which are readily changed into regular straight rods.

SUMMARY

The taxonomy of Lactobacillus bifidus is briefly discussed. Evidence is cited to show that the variation in morphology and in sensitivity to oxygen, regularly found in this bacterium, does not permit creation of distinct variants or subspecies. It is proposed that the current description of L. bifidus (L. bifidus (Tissier) Holland in Bergey's Manual) should be amended in certain paragraphs.

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SALMONELLA TYPHOSA VERSUS SALMONELLA TYPHI

A Request for an Opinion

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In studying the early literature in preparation for the next edition of Bergey's Manual, a solution has been found for a problem in bacteriological nomenclature that has puzzled many of us. When the Salmonella Subcommittee of the International Committee on Bacteriological Nomenclature (1), published their first report, they used Salmonella typhi (Schroeter) Warren and Scott as the name of the typhoid fever organism. The same specific epithet was also used in the first three editions of Bergey's Manual in the binomial Eberthella typhi (Schroeter) Buchanan.

C.D. Kelly, in reviewing early literature in preparation for the 4th edition of the Manual, discovered that Zopf (2) had given the name Bacillus typhosus to Eberth's typhoid bacterium before Schroeter (3) named it Bacillus typhi. For this reason the specific epithet "typhosa" has been used in the fourth, fifth, and sixth editions of the Manual. Zopf's 1885 name was validly published prior to the publication of Bacillus typhi Schroeter, 1886 and Bacillus typhi abdominalis Flügge, 1886 (4). However, some have not used the specific epithet "typhosus" because Klebs (5) had given the name Bacillus typhosus in 1880 to an organism that he isolated and described as the cause of typhoid fever. Subsequently it was shown that the organism described by Klebs was not the typhoid fever organism and that it was different from the true typhoid bacterium described first by Eberth (6) and later by Gaffky (7).

With the placing of the typhoid bacterium in the genus Salmonella in the 6th edition of Bergey's Manual with the specific epithet "typhosa" while the Enterobacteriaceae Subcommittee that has replaced the Salmonella Subcommittee continues to use Salmonella typhi, there has been a general request that the problem of the priority of these names be investigated so that there would be harmony in the nomenclature used in bacteriological literature.

In preparation for the next edition of Bergey's Manual, another review was made of the early literature describing

the typhoid organism whereupon it was discovered that the first name given by Zopf to Eberth's typhoid organism was Bacterium typhosum. This was validly published in the Anhang to the second edition of his Die Spaltpilze published in 1884, p. 90 (8). It had been overlooked because this binomial does not appear in the index. As both the organism and the binomial are different from the organism and the binomial as recorded by Klebs, there can be no question but that Zopf's Bacterium typhosum has priority over Schroeter's Bacillus typhi. It is proper to use not only Bacterium typhosum Zopf as the name for the typhoid bacterium, but also Salmonella typhosa (Zopf) White, Eberthella typhosa (Zopf) Weldin or any other suitable binomial except Bacillus typhosus Zopf. The latter binomial is an illegitimate homonym of Bacillus typhosus Klebs. Klebs' organism has never been placed in any genus but Bacillus and probably never will be as it was soon realized that it was not the true typhoid organism.

A number of papers have been reviewed in the search to determine who first used the binomial Salmonella typhosa. Thus far, no author has been found who used it earlier than White (9) in 1930. Apparently when it is desired to give a record of the origin of the binomial Salmonella typhosa it should be written Salmonella typhosa (Zopf, 1884) White, 1930.

When a preliminary draft of this manuscript was presented to Dr. R.E. Buchanan, Chairman of the Judicial Commission on Bacteriological Nomenclature, he raised the question whether Flügge did not publish his name, Bacillus typhi abdominalis, for the species earlier than 1886. Therefore a search was made for a copy of the first edition of Flügge's book (10) which was found to form a part of a larger Handbuch by v. Pettenkofer and v. Ziemssen. In the text, Flügge writes, p. 133: "Beim Typhus abdominalis fand Eberth (Lit. 280) unter 23 untersuchten Fällen 12 mal, später unter 17 Fällen 6 mal, in Lymphdrüsen und Milz kurze Stäbchen, an den Enden leicht abgerundet, verschmälerten Ovoiden gleichend; zuweilen waren Sporen in den Bacillen sichtbar." In no place in the text does he use anything other than a casual name for the Eberth organism; and the same statement holds true for all of the papers written on this topic by Eberth and by Gaffky.

However, Flügge copies Eberth's figure of his organism on the same page with the caption "Bacillus typhosus (?) (Nach Eberth)". It is not clear why Flügge placed the (?)

after this binomial, but the probability seems to be that he questioned whether Bacillus typhosus, the name that had been suggested previously for the typhoid organism by Klebs, was the correct name to use for Eberth's organism, the organism shown in his figure. Certain it is that when Flügge published the second edition of his book under the title Die Mikroorganismen, he did not use Bacillus typhosus for Eberth's organism, but proposed the new name Bacillus typhi abdominalis for it. It should be noted that while Flügge's name is composed of three words it is in reality a binomial as defined in the Botanical and Bacteriological Codes of Nomenclature. If used as a binomial it should be either hyphenated or, preferably, written as Bacillus typhiabdominalis.

The use by Flügge in 1883 of Klebs' name as a questionable name for the Eberth organism apparently does not in any way affect the legitimate status of the validly published Bacterium typhosum Zopf, 1884.

It is hoped that now that the story of the name for this typhoid organism is known, not only the Enterobacteriaceae Subcommittee, but also all others who have occasion to use a binomial for the typhoid organism will use it in the correct form.

Request for an Opinion

The Judicial Commission is requested to issue an Opinion determining the acceptability of Bacterium typhosum Zopf 1884 as the first legitimate name given to the causal organism of typhoid fever (typhus abdominalis), and the consequent legitimacy of the specific epithet in Salmonella typhosa (Zopf 1884) and White 1930.

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ON VIRUS NOMENCLATURE

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Dr. C.H. Andrewes (1955) has reported in this Bulletin on the results of a survey of views expressed by a representative group of animal and plant virologists on the question of virus nomenclature. From replies obtained to question (v) "do you favour a Linnaean System (a) as soon as possible, (b) ultimately, but not now, (c) not now, matter to be reviewed periodically, (d) not at all" only eight out of seventy-five of those who answered were in favour of (d).

Approximately 90 per cent of those who expressed their interest in the taxonomy of viruses, had at least a leaning towards the Linnaean System. However, forty-five out of the one hundred and twenty approached did not answer the questionnaire at all. Furthermore, the selection of the one hundred and twenty was made, according to Dr. Andrewes, "as representative as possible both on a geographical basis and on the basis of known or suspected opinions of the people concerned." The selection was not at random. Had it been so, the proportion not answering the questionnaire would probably have been higher.

It is not the author's intention to analyze and criticize the approach adopted by Dr. Andrewes but merely to use the information obtained by his survey to emphasize that the majority of workers, not only in the virus but also in the bacterial field, are not interested in taxonomic matters. Even amongst those who do express their opinions some do so solely in defence of a terminology to which they have become accustomed and without regard to the dictates of internationally accepted rules of nomenclature. It is therefore pertinent to ask what is, in fact, the immediate aim of the virologist? Is it to provide a nomenclature and classification for viruses or is it to ensure that when a certain virus is being discussed the virologists are all referring to the same thing? There is a subtle difference between the two and the latter can be quite simply resolved without resort to the former.

Anyone acquainted with developments in the bacterial field

is only too well aware that rules of nomenclature are not substitutes for sound means for identification. No matter how often the name of a bacterium may be changed, its stable characteristics, and hence the means by which it is identified, remain the same. What little stability has been achieved in the bacterial field has been due, not to mere agreement on a name, but on an agreement on the means of identification. This fact must be equally true in the virus field. Would it not suit the purpose of the virologist if, for the present, as an aid in the attempt to allocate names to virises, a move was made to draw up a mechanical scheme for their identification? The fact that different viruses are recognized, whether it be on differences in the characteristics of the virus particles themselves or in the pathological conditions they cause, must justify the statement that means exist for their separation. A mechanical scheme could set out exactly those steps which are taken at present to identify each known animal or plant virus or virus disease. This would take the form of a number of keys probably based on the hosts, the latter being referred to by their Linnaean binomials. At the termination of each branch of a key, not one, but all the names applied to a single virus could be placed. The list could include a general interpretation in several common languages. Such a proposal would have the following advantages:

(i) It would make clear the premises upon which any proposed name is based.

(ii) It would make clear the several names which have been applied to the one virus and at the same time standardize the several linguistic expressions of the same statement.

(iii) It would satisfy the requirements of those virologists whose immediate interest is virus identification and not nomenclature.

(iv) It would serve the immediate requirements of all virologists from a nomenclatural viewpoint and may stimulate a number to take an interest in virus taxonomy by providing a common ground for discussion.

(v) It would render unnecessary any immediate attempt to introduce a universally acceptable system of nomenclature.

(vi) It would clarify the state of our knowledge of those viruses or virus diseases which at present are too poorly understood to merit even the non-Linnaean binomials recommended at the Rome Congress (1953).

What of the Linnaean System?

Within English speaking countries, Sus scrofa Linnaeus is known as a pig in Australia, a hog in America and a swine elsewhere. It requires no imagination to perceive the chaos arising from the multilingual treatment of even a single virus such as that of swine influenza! Nevertheless, the use of this multiplicity of common names for the one virus is valid in mechanical keys to aid in the resolution of differences in terminology. Something must ultimately be done, however, to arrive at a single internationally acceptable name to replace them. It must also be in an internationally acceptable language. There appears to be no good reason why this should not be Latin.

It has quite justifiably been asserted that we know too little about the phylogenetical relationship of viruses to be embarking on the use of a Linnaean system of nomenclature. Does it necessarily follow that the use of a Latin binomial infers a phylogenetical relationship? Van Niel has pointed out that Cohn, in using the Latin binomial in naming species and genera in bacteria, regarded the latter as form genera. If virologists wish to preserve the scientific dignity of the Linnaean binomial it should be sufficient to suggest--in a mechanical system--a latinized name to replace the multiplicity of common names. Such names could be used in international publications until such time as virologists consider it opportune to replace them with Linnaean binomials.

A proposal of this nature was submitted by the Virus Subcommittee of the International Nomenclature Committee at the 6th International Congress of Microbiology at Rome in September, 1953.

It should, however, be emphasized that resolution of the present differences regarding the use of latinized names for viruses is really a minor matter. The real problem lies in the realm of virus classification, that is, the determination

of biological relationships amongst viruses. This must, of course, be resolved before any generic names can be supplied. Because we have little information on this subject, the majority of virologists are opposed to the introduction of a classification such as that proposed by Holmes. Whilst one must agree with those who hold this view, the author supports the contention of one prominent virologist that something must be done to sort out the mass of information which has accumulated. It is quite possible to do this by the use of the mechanical scheme outlined in preceding paragraphs.

One must add a word, however, in defence of Holmes, In the author's own experience in endeavouring to produce a mechanical system for the generic identification of bacteria (Skerman 1949) of which an expanded edition is being prepared for inclusion in the 7th edition of Bergey's Manual, it is dangerous to attempt to generalize over the whole of the virus field on the basis of experience gained in only a limited aspect of it. Only Holmes and others who have attempted the task can readily appreciate the problems involved in trying to draw up a general virus classification. Irrespective of what views may be held of the Holmes classification, there can be no doubt that it has precipitated a long overdue consideration of the problem.

On the matter of compromise.

From replies received to the first three questions in Andrewes questionnaire, it is apparent that the majority of virologists favour treatment of all viruses along parallel lines and some compromise between diverging views on virus nomenclature.

Taxonomic matters should not be taken too seriously. It is true that there is much to be gained from the study of the biological relationships within the animal and plant kingdoms but it is equally true that our efforts in this direction are limited entirely by the tools of our profession. In the bacterial field, the history of classification is in fact a history of technical development. With changes in technique, we have had added information which has led to changes in our conception of bacterial relationships. What may be regarded now as varieties of a species may possibly, years hence, be so subdivided within themselves as to be raised to generic rank. Dogmatism in taxonomy is therefore pointless. If we

are to achieve anything of use to the present generation of scientists, we must treat taxonomic matters in a more philosophical way. The only answer is compromise.

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THEODOR THJØTTA
1885 -- 1955

An Appreciation

Theodor Thjötta, professor of medical bacteriology and serology at Oslo University, died suddenly on June 13, 1955, only 4 days before his 70th birthday. He was born in Sandnes in southwestern Norway in 1885. Originally he had planned a career as a naval officer, and in his early youth he sailed the seven seas in sailing ships for three years. Fortunately, one might say, he lost a part of a finger through an infection, and according to the strict requirements of those days he was rejected by the navy as medically unfit.

The same accident turned his interest towards medicine, and as soon as his ship called at a European port, he went home to complete his school education and to study medicine. In the course of his studies he went to sea again as a drift-net fisherman in the North Sea. Later on he was doctor on a whaling expedition to West Africa for two seasons. Throughout his life he remained something of a sailor at heart, and by a strange coincidence the book he had been reading on the night he died was entitled "A sailor goes ashore".

While in West Africa he had to tackle a bad epidemic of bacillary dysentery, and this made him interested in bacteriology.

From 1915 he studied bacteriology and pathology in Bergen under the well known pathologist Magnus Haaland, and he completed his training by a couple of years at the hospital for infectious diseases. In Bergen he mainly worked with dysentery bacilli, the theme of his thesis, and he arrived at a system of classification which differs little from the one in use today. In 1919 he was appointed chief of the newly established bacteriological laboratory of the Norwegian army, but first he went to the U.S.A. for further studies of bacteriology, mainly of the respiratory tract, at the Hospital of the Rockefeller Institute for Medical Research. In 1920 he returned to his laboratory in Oslo, where he worked until he was appointed professor of bacteriology and serology at Oslo University in 1935.

Thjötta was a very active man with a strong will and abundant energy. Throughout his life he maintained a keen interest in research and published a large number of papers and

books covering all aspects of medical microbiology. Only his more outstanding contributions to science can be mentioned here; his study of the dysentery bacilli (1917), his studies in collaboration with Avery on the growth requirements of the influenza bacilli (1921-24), his studies on tularemia in Norway (1930-31) with the first isolation of the organism in this part of the world, his studies on the bacteriology of fish (1942), on Leptotrichia (1944), on Weil's disease in Norway (1941) and others too numerous to mention.

All his papers were written in a clear and stimulating style, and always contained observations of scientific interest. Particularly in the early years, his papers had the double purpose of reporting new scientific results and of educating the Norwegian physicians, many of whom knew next to nothing about modern bacteriology and its practical value.

He was a gifted and inspiring teacher. Before his day, bacteriology in Norway was in a rather primitive stage, and the medical students learned only the bare and dry facts of classical bacteriology. Thjötta radically modernized the tuition and activated the students by practical courses, demonstrations, and discussions. He published a textbook covering the whole field of medical microbiology. The young physicians working under him also were inspired to great scientific effort, resulting in a very large number of scientific papers as well as ponderous theses. Furthermore, he managed to pursuade another man of the sea, Captain W. Wilhelmsen, owner of the W. Wilhelmsen Shipping Company, to donate funds for the building of a magnificent new bacteriological institute, which the university was very badly in need of.

He also was active on the international level, and took part in numerous congresses. He was a member of the International Committee on Bacteriological Nomenclature, of the Judicial Commission (class of 1959), of the Botanical Committee on Bacteria, the Subcommittee on the Family Neisseriaceae, and of the American Society of Bacteriologists (lately corresponding member). Furthermore, he was elected member of the Norwegian Academy of Science in Oslo (president of the Mathematics-Science Class in 1938), of the Copenhagen Medical Society, and of the Swedish Physicians' Society. He acted as one of the Norwegian editors of the Acta Pathologica et Microbiologica Scandinavica for many years.

For his great services to Norwegian bacteriology he received the Fridtjof Nansen award from the Norwegian Academy of Science in Oslo in 1950, and a few months before his death he was created a commander of the St. Olav Order.

His greatest achievement may be that he successfully acted as a pioneer in a country which was "underdeveloped" in his field, and introduced all the new ideas which were developing. Thanks to his efforts bacteriology has a much better standing now in his country than it had when he started his work.

He was a very striking and colorful personality, a man of great gifts and great energy, and his life was busy and fruitful. His untimely death is regretted by many colleagues scattered throughout the world.

S. D. Henriksen

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Coh
Cow
4

Cra
Cru

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Dav
De
De
De
De
Der
De
Die
Dou
Dub
Dun

Adams, M.H. 50
Ainsworth, G.C. 45, 51, 96
Allen, Oscar N. 40, 51
Allison, V.D. 47
Andrewes, C.H. 37, 41 48, 49, 79
Arnaudi, Carlo 38
Asai, Toshinobu 38
Aschner, M 37

Babudieri, B. 44
Ball, M. 44
Bang, F.B. 49
Bawden, F.C. 51, 79
Bedson, S.P. 50
Bennett, C.W. 51, 79
Bensted, H.J. 43, 46
Bergold, G.H. 49, 50
Bessemans, A. 44
Bird, F.T. 50
Bitancourt, A. 34, 35
Borg, Peterson C. 44
Boyd, J.S.K. 43
Boycott, J. 5
Bradford, W.L. Jr. 61
Branham, Sara E. 46
Breed, Robert S. 7, 21, 33 51, 61, 165
Brisou, J. 70, 85, 89
Broom, J.C. 44
Brown, J. Howard 40
Bruner, D.W. 43
Bruynoghe, Richard 34
Buchanan, R.E. 40, 41, 51
Buchwald, N. F. 36
Buddle, M.B. 38
Burnet, Sir M. 49, 50

Carlquist, P.R. 43
Carrion, A.L. 45
Castellani, Aldo 38
Chain, E.B. 46
Christiansen, M. 36
Ciferri, R. 38, 45, 96
Clark, Francis E. 111
Cohen, Sophia M. 46
Cowan, S.T. 33, 42, 43 44, 45, 47, 51, 62, 63, 65 97, 133, 141
Craigie, J. 50
Cruz, Oswaldo 35

Da Rocha Lagoa, F. 35
Davis, Gordon E. 44, 107
De Almeida, F. 45
De Area Leao, A.E. 45
De Azevedo, Fraga 44
De Rossi, Gino 38
Derrick, E.H. 44
De Salles Gomes, L. 44
Dienes, L. 68, 69, 71, 85
Douglas, S. Hilda 5
Dubos, R.J. 46
Duncan, J.T. 45

Eaton, M.D. 75
Eddy, B. 47
Edward, D.G. 67, 74, 75, 85
Edwards, P.R. 43, 151
Eisler-Terramare, M. 34
Emmons, C.W. 45
Evans, James B. 61
Ewing, W.H. 43, 105

Falco, A. 39
Ferguson, W.W. 43
Ferris, A.A. 47
Fitzgerald, W.A. 67
Fredette, V. 42
Frei, W. 40
Freundt, E.A. 67, 85
Fry, R.M. 47

Gaby, W.L. 153
Gard, Sven 39, 49
Gear, J.H.S. 50
Gibson, T. 37, 61
Gillespie, E.H. 2
Giovanardi, A. 43
Gonnert, R. 50
Goodpasture, E.W. 50
Gordon, Ruth E. 46
Graf-Marin, Alberto 35
Grasset, E. 40
Grimes, Michael 36
Guida, V.O. 44
Gyllenberg, H.H.G. 36, 161

Hagan, W.A. 46
Hammon, W. McD. 49
Hanks, John 46
Hansen, P.A. 47
Hauduroy, Paul 36, 46
Haupt, Herbert 37
Henneberg, G. 49
Henriksen, S. Dirk 39
Herick, William Van 75
Holt, H.D. 1
Horgan, E.S. 50
Hormaeche, E. 40, 43
Horowitz-Wlassowa 40
Hosakawa, S. 68
Hucker, G.J. 62, 63
Hülphers, Gustav 39
Hughes, K.M. 50

Ionescu-Mihaiesti, C. 39
Issatchenko, B. 40

Janke, Alexander 34
Jensen, K.A. 46

Kästli, P. 40
Kauffmann, F. 36, 42, 47
Kawamura, H. 68
Kirschner, L. 38
Klieneberger, E. 68, 69, 85
Klinge, Karl 74
Kluyver, A.J. 38, 41
Kohler, E. 51
Kojima, Sabura 38
Kolochine-Erber, B. 44
Kominami, Kiyoshi 38

Lancefield, R. 47
Lanjouw, J. 52
Ledingham, J.C.G. 68, 70
Legezynski, St. 39
LeMinor, L. 43
Lepine, P. 37, 49
Lessel, E.Z. Jr. 7, 21
Limasset. P. 51
Lochhead, A.G. 35, 115
Lund, E.M. 48
Lwoff, A. 37, 41

McCoy, E. 42
McKay, K.A. 74, 75
McWhorter, Alma C. 151
McWhorter, F.P. 51

MacClung, L.S. 42
MacKinnon, Juan E. 45
Magrasse, 49
Malek, Ivan 36
Manninger, R. 37
Markham, F.S. 75
Markoff, W. 35
Mattick, A.T.R. 48
Maxsted, W.R. 48
Meyer, K.F. 44
Miles, A.A. 37, 42
Miller, C. Philip 47
Mocquot, G. 48
Mooser, H. 40
Morin, E.J. 35
Morton, H.E. 67, 69
Munch-Peterson, E. 34
Muniz, Julio 44
Murray, E.G.D. 33, 35 42, 47
Mustakallio, E. 36

Nagington, J. 2
Negroni, P. 45
Nelson, J.B. 75
Nickerson, W.J. 45
Nicolle, P. 50
Nilsson, R. 39
Niven, C.F. Jr. 48, 61
Nowack, J. 68

Olin, G. 43
Olitsky, P.K. 49
Oliveira, Candido de 39
Ørskov, J. 36, 49, 68, 69
Oxford, A.E. 131

Pakula, R. 48
Pattison, I.H. 48
Paul, J.R. 50
Pelczar, M.J. 47
Penha, Adolpho M. 35
Penido, J.C.N. 35, 41
Penso, G. 38, 46
Pentinnen, E. 36
Petroff, T. 35

Prentice, I.W. 51
Prévot, A.R. 37, 42, 47
Price, Audrey U. 1
Przesmycki, F. 39

Rake, G. 49, 50, 79
Ramsbottom, J. 37, 41, 52
Raska, Karel 36
Rauss, K. 43
Redaelli, P. 45
Reed, Guilford B. 35, 46
Renaux, Ernest 34, 41
Rennella, E. 44
Richardson, G.M. 63
Rivers, T.M. 49
Ross, H.E. 42
Rubbo, S.D. 34
Ruys, A. Charlotte 38

Sabin, A.B. 49, 70, 73
74, 86, 87
San Miguel, Julio 35
Santos, Maria 44
Schlossberger, H. 45
Seelemann, M. 37, 48
Seeliger, H. 37, 43
Segretain, G. 37
Sermoli, R.E.G. 52
Shaw, Constance 62, 63, 65
Sherman, J.M. 48
Shoetensack, H.M. 69
Skadhauge, K. 48
Skerman, V.B.D. 34, 169
Skinner, C.E. 46
Smiles, J. 68
Smit, Jan 38
Smith, P.F. 67
Sordelli, A. 34, 41
Soriano, Santos 34
Spray, H.S. 42
Stableforth, A.W. 48
Stapp, C. 37
Steinhaus, E.A. 50
Stitt, Jennifer M. 62, 63
St. John-Brooks, R. 33, 47
Stuart, C.A. 43
Szylvini, 34
Szymanoski, F. 39

Tang, F.F. 68
Taylor, Joan 5, 43, 75
Terrier, Ch. 40
Thaysen, A.C. 35, 42
Thjøtta, Th. 39, 41, 47
52, 175
Thung, T.H. 51
Tulasne, Robert 68, 70
85, 89
Turner, A.W. 43, 68, 87, 88

Updyke, E. 48

Vaccaro, Hugo 36
Van der Hoeden, J. 45
Van Eseltine, Wm. P. 53
Van Niel, C.B. 40, 41
Van Riel, J. 45
Van Rooyen, C.E. 35
Van Slogerteren, E. 51
Van Thiel, J. 45
Vartiovaara, J. 33, 36
Verlinde, J.D. 38
Von Magnus, H. 50
Voss, J. 39

Wahl, R. 48
Watkins, J.F. 5
Weinberger, H.J. 68, 85
Wells, A.Q. 46
Westerdijk, Johanna 46
Whitehead, H.R. 38
Wiesmann, W. 44
Wikén, Torsten 33, 42
46, 48, 52
Wilhelm, O. 36, 45
Williams, R.E.O. 47, 48, 65
Wilson, A.T. 48
Wilson, G.S. 37
Wolff, J.W. 38, 44
Wong, S.C. 75
Wroblewski, W. 68, 87
Wyatt, G.R. 50
Yamazaki, Izue 46
Zischka, R.L. 34
Zotta, G. 39

Actinomyces 14, 72
Actinomycetaceae 70
Actinomycetales 70
Aerobacter 146, 147
  aerogenes 97, 102, 103
  cloacae 97, 102, 103
Ancyromonas
  ruminantium 8
Annulomyces 17
Anulomyces 87
  agalaxiae 13, 16, 73
  agalactiae 16
Anulomycetaceae 70
Anulomycetales 70
Arthrobacter 83, 84, 111
  aurescens 112, 113
  globiforme 83, 112, 113
    116, 117
  ureafaciens 111, 112, 113
Asterococcus 13, 14, 15
    18, 19, 72, 88
  canis 69, 73
  mycoides 13, 18
    19, 73, 87
    var. capri 73
Asteromyces 16, 87
  mycoides 16, 17
  peripneumoniae 13
    bovis 15, 16
Azotobacter 125

Bacillus
  helvolus 115, 116, 118
  typhi 165, 166
    abdominalis 165
    166, 167
  typhosus 165, 166. 167
Bactepneumoniales 85
Bacterium 22
  aerogenes 102
  globiforme 116
  helvolum 115, 116, 118
  nitrobacter 23, 29
  rauricum 22
  typhosum 166, 167
Bacterius 22
Biochemical
  hippurate test 97
  gluconate test 141
Borrelia 7, 17
  neotropicalis 108, 109
  venezuelensis 108, 109
Borrelina 121, 126, 127
  bombycis 124, 126
Borrelinaceae 126
Borrelomyces 15, 17, 18, 19
    70, 72, 87, 88
  agalactiae 17
  bovigenitalium 74
  gallinarum 75
  mycoides 17
  peripneumoniae 13, 16, 17
Borrelomycetaceae 70, 71
Borrelomycetales 17, 67
    70, 71, 88
Bovimyces agalactiae 17
  mycoides 17
  pleuropneumoniae 13, 17
Brevibacteriaceae 118
Brevibacterium
  helvolum 115, 118
  linens 118

Canomyces 17
Capromyces 17
Chlamydomonas
  bombycis 126
Chlamydozoaceae 122, 124
Chlamydozoon 121, 122
    123, 124, 126, 127
  bombycis 122, 124
    125, 126, 127
  trachomatis 122, 123, 127
Chytridiaceae 14
Cloaca 103
  cloacae 101, 102
Clostrillum 21
Clostrinium 21
Coccobacillus 14, 15, 72
  avicidus 14
  mycoides
    peripneumoniae 13, 14
Colesiota 122
Commissions,
  membership 33
Committees,
  membership 33
Corynebacterium 116, 117
  creatinovorans 111
    112, 113
  helvolum 115, 116
    117, 118
  ureafaciens 111, 112, 113

Drepanospira muelleri 125

Eberthella typhi 165
  typhosa 166
Enterobacteriaceae 102

Escherichia
  coli 98, 99, 101, 102
  freundii 102

Flavobacterium 117
  helvolum 115, 118

Gaffkya tetragena 53

Haemophilus gallinarum 76

Klebsiella 98, 99, 102
    146, 147
  aerogenes 101, 102
  ozaenae 99, 101, 102
  pneumoniae 99, 101
    102, 103
Kurthia 118

Lactobacillus bifidus
    161, 162, 163
Lactobacillus (cont.)
  parabifidus 161, 162
  bifidus
    var. pennsylvanicus
    162
Linneaean system 171

Micrococcus 53, 54, 58
    59, 61, 63, 65, 133
  agilis 64
  candicans 64
  candidus 64
  caseolyticus 55, 64
  citreus 55, 64
  conglomeratus 55, 64
  denitrificans 64
  epidermidis 55, 64
  flavus 64
  freudenreichii 64
  luteus 58, 59, 61
    62, 64, 65
  lysodeikticus 64
  nitrosus 28
  pyogenes 64
    var. albus 55
    var. aureus 55
  rhodochrous 64
  roseus 64
  rubens 64
  tetragenus 53
  ureae 64
  varians 64
Micromyces 14, 15, 17
  hoffmanni 14
  peripneumoniae bovis
    contagiosae 13, 14
  zygogonii 14
Miyagawanella 122
Mollicutales 89
Morator 126
Müllerina parmecii 125
Murimyces
  arthritidis 73
  pulmonis 73
Musculomyces
  arthrotropicus 73
  histotropicus 73
  neurolyticus 73
Mycoplasma 16, 17, 18, 72
    73, 87, 88, 89
  agalactiae 68, 69, 73, 90
  arthritidis 68, 73, 91
  bovigenitale 90
  bovigenitalium 73
  canis 73, 74, 90
  fermentans 73, 90
  gallinarum 73
  hominis 73, 90
  laidlawii 67, 69, 73, 91
  maculosum 73, 90
  mycoides 15, 67
    69, 73, 89
    var. capri 69, 73, 90
  neurolyticum 73, 91
  peripneumoniae 13, 15

Mycoplasma (cont.)
pulmonis 69, 73, 91
salivarium 73, 90
spumans 73, 74, 90
Mycoplasmataceae 89
Mycoplasmatales 73
Mycromyces 15

Nitrobacter 23, 27, 28, 29
nitrobacter 23, 29
oligotrophum 29
polytrophum 29
winogradskyi 23, 30
Nitromonas 27, 28, 30
europaea 28
javanensis 28
Nitrosococcus 23, 27
28, 29, 30
americanus 28
europaea 28
nitrosus 28, 29, 30
Nitrosomonas 23, 27, 28, 30
europaea 30
Nocardia 70

Ornithodoros
rudis 107, 108
talaje 107, 108
venezuelensis 108
Oscillaria 9

Parachromatium 125
Paramycetales 70
Paramycetes 70
Parasitaceae 70
Pasteurella 14, 71
Pleuropneumoniales
70, 85, 89
Proteus 102, 155
Pseudomonas 153
aeruginosa 141, 153, 154
155, 156, 157, 158, 159
aptata 156
chlororaphis 155
fluorescens 153, 155, 157
fragi 155, 157
graveolens 155, 157
hydrophila 155, 157
marginale 156

Pseudomonas (cont.)
mildenbergii 155, 157
mucidolens 155
oleovorans 157
ovalis 155
pavonacea 157
polycolor 156
putida 155, 157
putrefaciens 155, 157

Rickettsia
trachomae 123, 127
trachomatis 122, 123, 127
Rickettsiales 86, 122, 123

Salmonella 102, 151, 152, 155
designation of new
serotypes 105, 106
abortus-equi 5, 152
berlin 2
boecker 106
brazzaville 106
bredeney 2
carrau 3
colindale 1, 3
deversoir 5, 6
eschweiler 151
gallinarum 151
gaminara 2
hull 106
illinois 151
isangi 2
kapemba 106
kingabwa 106
landau 106
manila 106
minneapolis 106
newport var.
puerto rico 2
oranienburg 2
panama 152
paratyphi 5, 151
portland 151, 152
rubislaw 2
seattle 151, 152
tel hashomer 151
thompson 2, 151
typhi 165
typhosa 165, 166, 167

Sapromyces
laidlawii 73
Schizosaccharomyces
ovis 132
Selenomastix 7, 9, 10, 131
ruminantium 9, 10, 132
Selenomonas 7, 8, 9, 10, 21
23, 131, 132
palpitans 7, 9, 131
ruminantium 8
sputigena 8, 9, 10, 24
Shigella 102, 155
flexneri 152
Spirillaceae 9
Spirillum
sputigenum 7, 8, 10, 24
Spirochaeta
neotropicalis 109
Spirochaetaceae 17
Staphylococcus 53, 54, 58
59, 61, 62, 63, 65, 133, 135
albus 134
aureus 58, 62, 133
134, 135
flavus 134
pyogenes 62, 63, 65
133, 134, 135
albus 134
aureus 133
Streptococcus
pyogenes 135
Subcommittees,
membership 33

Testudo graeca 5
Treponema venezuelense
108, 109

Virales 86, 123
Virus nomenclature 79, 169

Xanthomonas 153

Zygogonium 14

182
73
132
0, 131
0, 132
10, 21
1, 132
9, 131
8
10, 24
2, 155
152
9
10, 24
109
17
54, 58
3, 135
134
2, 133
4, 135
134
3, 65
4, 135
134
133
135
35
5
nse
8, 109
6, 123
9, 169
153
14